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Term: L4 same Fusion

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	DB=USPT,PGPB,JPAB,EPAB,DWPI; PLUR=YES; OP=ADJ		
L7	L4 same Fusion	16	L7
L6	L4 same (chimer$$)	15	L6
L5	L4 same (hybrid adj enzyme)	0	L5
L4	l1 same (synthesi$$$ or biosynthesi$$$)	1722	L4
L3	l1 same (synthe$$$ or biosynthe$$$)	1791	L3
L2	L1 same (synthe$$$ or biosynthe$$$ or manufactur$$$ or produc$$$)	5639	L2
L1	(sugar adj nucleotide)or oligosaccharide	14659	L1

END OF SEARCH HISTORY

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L7: Entry 4 of 16 File: USPT Sep 4, 2001

US-PAT-NO: 6284494
DOCUMENT-IDENTIFIER: US 6284494 B1

TITLE: Methods and compositions for synthesis of oligosaccharides using mutant glycosidase enzymes

DATE-ISSUED: September 4, 2001

INVENTOR-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY
Withers; Stephen G.	Vancouver			CAX
MacKenzie; Lloyd	Vancouver			CAX
Wang; Qingping	Kirkland			CAX

ASSIGNEE-INFORMATION:

NAME	CITY	STATE	ZIP CODE	COUNTRY	TYPE CODE
The University of British Columbia	Vancouver			CAX	03

APPL-NO: 9/091272 [PALM]
DATE FILED: September 29, 1998

PARENT-CASE:
This application is a U.S. National Phase, filed under 35 USC .sctn. 371, of PCT/CA96/00841, which is a continuation-in-part of U.S. patent application Ser. No. 08/571,175 filed Dec. 12, 1995, now U.S. Pat. No. 5,716,812.

PCT-DATA:

APPL-NO	DATE-FILED	PUB-NO	PUB-DATE	371-DATE	102(E)-DATE
PCT/CA96/00841	December 12, 1996	WO97/21822	Jun 19, 1997	Sep 29, 1998	Sep 29, 1998

INT-CL: [7] C12 P 19/44, C12 P 19/12, C12 N 9/24, C12 N 9/26, C12 N 9/42

US-CL-ISSUED: 435/74; 435/100, 435/200, 435/201, 435/209
US-CL-CURRENT: 435/74; 435/100, 435/200, 435/201, 435/209

FIELD-OF-SEARCH: 435/74, 435/100, 435/200, 435/201, 435/209, 435/440

PRIOR-ART-DISCLOSED:

U.S. PATENT DOCUMENTS

Search Selected | Search ALL

	PAT-NO	ISSUE-DATE	PATENTEE-NAME	US-CL
☐	4918009	April 1990	Nilsson	435/73
☐	5246840	September 1993	Nilsson	435/101
☐	5372937	December 1994	Nilsson	435/74

FOREIGN PATENT DOCUMENTS

FOREIGN-PAT-NO	PUBN-DATE	COUNTRY	US-CL
0226563	June 1987	EPX	
87/05936	October 1987	WOX	
89/09275	October 1989	WOX	
94/29477	December 1994	WOX	
95/18864	July 1995	WOX	
95/18232	July 1995	WOX	

OTHER PUBLICATIONS

Withers et al., "Mechanistic Comsequences of Mutation of the Active Site Nucleophile GLU 358 in Agrobacterium .beta.-Glucosidase" Biochemistry 31: 9979-9985 (1992).
Trimbur et al., A .beta.-Glucosidase from an Agrobacterium sp.: Structure and Biochemistry in ACS Sympsium Series (1992) pp. 42-55.
Gebler et al., "Substrate-Induced Inactivation of a Crippled .beta.-Glucosidase Mutant: Identification of the labeled Amino Acid and Mutagenic Analysis of Its Role", Biochemistry 34: 14547-14553 (1995).
Wang et al., "Identification of the Acid/Base catalyst in Agrobacterium faecalis .beta.-glucosidase by kinetic analysis of mutants" Biochemistry 34: 14454-14562 (1995).
Wang et al., "Substrate-assisted Catalysis in Glycosidases" J. Amer. Chem. Soc. 117: 10137-1-138 (1995).
Witt et al., "6-Phospho-.beta.-galactosidases of Gram Positive and 6-phospho-.beta.-glucosidase B of Gram-Negative bacteria: comparison of structure and function by kinetic and immunological methods and mutageneisis of the lacG gene of Staphyloccous aureus" Protein Engineering 6: 913-920 (1993).
Nikolova et al., "Transglycosylation by Wild Type and Mutants of a .beta.-1,4-Glycosidase from Cellulomonas fimi (Cex) for synthesis of Oligosaccharides", Annals NY Acad. Sci. 799: 19-25 (1996).
Wang, et al. (1994) "Changing Enxymic Reaction Mechanisms by Mutagenesis: Conversion of a Retaining Glucosidase to an Inverting Enzyme", J. Am. Chem. Soc. 116:11594-11595.
Svensson, (1988) FEBS Letters 230:72-76.
Nagashima, et al. (1992) Biosci. Biotech. Biochem. 56:207-210.

ART-UNIT: 162
PRIMARY-EXAMINER: Slobodyansky; Elizabeth
ATTY-AGENT-FIRM: Oppedahl & Larson LLP

ABSTRACT:

Mutant glycosidase enzymes are formed in which the normal nucleophilic amino acid within the active site has been changed to a non-nucleophilic amino acid. These enzymes cannot hydrolyze disaccharide products, but which can still form them. Using this enzyme, oligosaccharides are synthesized by preparing a mixture of an .alpha.-glycosyl fluoride and a glycoside acceptor molecule; enzymatically coupling the .alpha.-glycosyl fluoride to the glycoside acceptor molecule to form a glycosyl glycoside product using the mutant glycosidase enzyme; and recovering the glycosyl glycoside product. Particular enzymes include a mutant form of Agrobacterium .beta.-Glucosidase in which the normal glutamic acid residue at position 358 is replaced with an alanine residue.

2 Claims, 3 Drawing figures

L7 ANSWER 20 OF 27 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:165227 CAPLUS
DOCUMENT NUMBER: 126:156479
TITLE: Manufacture of novel polyketides by expression of foreign polyketide synthase genes in a polyketide-synthesizing microbial host
INVENTOR(S): Khosla, Chaitan; Hopwood, David A.; Ebert-Khosla, Suzanne; Mcdaniel, Robert; Fu, Hong; Kao, Camilla
PATENT ASSIGNEE(S): Leland Stanford Junior University, USA; John Innes Centre
SOURCE: PCT Int. Appl., 132 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 7
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9640968	A1	19961219	WO 1996-US9320	19960605
W: AL, AM, AU, BB, BG, BR, CA, CN, CZ, EE, FI, GE, HU, IL, IS, JP, KG, KP, KR, LK, LR, LT, LV, MD, MG, MK, MN, MX, NO, NZ, PL, RO, SG, SI, SK, TR, TT, UA, UZ, VN, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
US 5712146	A	19980127	US 1995-486645	19950607
AU 9661575	A1	19961230	AU 1996-61575	19960605
AU 703920	B2	19990401		
EP 871760	A1	19981021	EP 1996-919168	19960605
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:

US 1995-486645	A	19950607
US 1993-123732	B2	19930920
US 1993-164301	B2	19931208
US 1994-238811	A2	19940506
WO 1996-US9320	W	19960605

OTHER SOURCE(S): MARPAT 126:156479

AB A method of manufg. novel and known polyketides by expression of the genes for polyketide synthetases (PKSs) in foreign hosts is described. In particular, a novel host-vector system is described which is used to produce polyketide synthases which in turn catalyze the prodn. of a variety of polyketides. The genes may be mutated to produced **enzymes** with altered properties leading to the formation of novel polyketides. The preferred host is a Streptomyces coelicolor CH999 in which the endogenous actinorhodin PKS gene cluster is replaced. The construction of genes for a no. of **chimeric** polyketide synthases is described. The structures of the novel polyketides synthesized by these **enzymes** are used to elucidate the mechanisms of polyketide synthesis by these **enzymes**. Data from these expts. were used to construct **chimeric** polyketide synthases designed to catalyze the formation of specific products. The construction of polyketide synthase

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(FILE 'HOME' ENTERED AT 14:29:27 ON 07 MAY 2002)

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:29:40 ON 07 MAY 2002

SEA	ENZYME
8844	FILE ADISALERTS
3380	FILE ADISINSIGHT
1771	FILE ADISNEWS
82992	FILE AGRICOLA
12494	FILE ANABSTR
19547	FILE AQUASCI
53313	FILE BIOBUSINESS
12742	FILE BIOCOMMERCE
642440	FILE BIOSIS
92228	FILE BIOTECHABS
92228	FILE BIOTECHDS
300806	FILE BIOTECHNO
169044	FILE CABA
99358	FILE CANCERLIT
798553	FILE CAPLUS
27932	FILE CEABA-VTB
1524	FILE CEN
6548	FILE CIN
10028	FILE CONFSCI
5084	FILE CROPB
5013	FILE CROPU
29199	FILE DDFB
38582	FILE DDFU
148103	FILE DGENE
29199	FILE DRUGB
371	FILE DRUGLAUNCH
72	FILE DRUGMONOG2
663	FILE DRUGNL
55034	FILE DRUGU
715	FILE DRUGUPDATES
3256	FILE EMBAL
627925	FILE EMBASE
163249	FILE ESBIOBASE
166	FILE FOMAD
1043	FILE FOREGE
31935	FILE FROSTI
45036	FILE FSTA
72053	FILE GENBANK
1860	FILE HEALSAFE
24725	FILE IFIPAT
187536	FILE JICST-EPLUS
1105	FILE KOSMET
171354	FILE LIFESCI
338	FILE MEDICONF
596249	FILE MEDLINE
16437	FILE NIOSHTIC

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          11537   FILE NTIS
           5790   FILE OCEAN
         422732   FILE PASCAL
           7244   FILE PHAR
             28   FILE PHIC
           5705   FILE PHIN
          30501   FILE PROMT
         367891   FILE SCISEARCH
             78   FILE SYNTHLINE
         286015   FILE TOXCENTER
         106258   FILE USPATFULL
            279   FILE USPAT2
          58240   FILE WPIDS
          58240   FILE WPINDEX
L1             QUE ENZYME
               ---------

     FILE 'CAPLUS, BIOSIS, EMBASE, MEDLINE, SCISEARCH, BIOTECHNO' ENTERED AT
     14:32:36 ON 07 MAY 2002
L2          57169 S L1 AND (FUSION) OR (DUAL(W)FUNCTION)
L3            275 S L2 AND GLYCOSYLTRANSFERASE
L4             10 S L3 AND NUCLEOTIDE(W)SUGAR
L5              5 DUP REM L4 (5 DUPLICATES REMOVED)
L6             35 S L3 AND CHIMER?
L7             27 DUP REM L6 (8 DUPLICATES REMOVED)
L8           2441 S (SUGAR(W)NUCLEOTIDE) OR OLIGOSACCAHRIDE
L9         895222 S L8 AND (SYNTHE?) OR (BIOSYNTHE?)
L10          1459 S L8 AND (SYNTHE? OR BIOSYNTHE?)
L11            27 S L10 AND (HYBRID(W)ENZYME OR CHIMER? OR FUSION)
L12            14 DUP REM L11 (13 DUPLICATES REMOVED)
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=> d l12 ibib ab 1-12

L12 ANSWER 1 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2002:308856 SCISEARCH
THE GENUINE ARTICLE: 538TL
TITLE: Combined **biosynthetic** pathway for de novo production of UDP-galactose: Catalysis with multiple enzymes immobilized on agarose beads
AUTHOR: Liu Z Y; Zhang J B; Chen X; Wang P G (Reprint)
CORPORATE SOURCE: Wayne State Univ, Dept Chem, Detroit, MI 48202 USA (Reprint); Neose Technol Ltd, Hursham, PA 19044 USA
COUNTRY OF AUTHOR: USA
SOURCE: CHEMBIOCHEM, (2 APR 2002) Vol. 3, No. 4, pp. 348-355. Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61, D-69451 WEINHEIM, GERMANY. ISSN: 1439-4227.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 46

ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Regeneration of **sugar nucleotides** is a critical step in the **biosynthetic** pathway for the formation of oligosaccharides. To alleviate the difficulties in the production of **sugar nucleotides**, we have developed a method to produce uridine diphosphate galactose (UDP-galactose). The combined **biosynthetic** pathway, which involves seven enzymes, is composed of three parts: i) the main pathway to form UDP-galoctose from galactose, with the enzymes galactokinase, galactose-1-phosphate uridyltransferase, UDP-glucose pyrophosphorylase, and inorganic pyrophosphotase, 11) the uridine triphosphate supply pathway catalyzed by uridine monophosphate (UMP) kinase and nucleotide diphosphate kinase, and iii) the adenosine triphosphate (ATP) regeneration pathway catalyzed by polyphosphate kinase with polyphosphate added as an energy resource. All of the enzymes were expressed individually and immobilized through their hexahistidine tags onto nickel agarose beads ("super beads"). The reaction requires a stoichlometric amount of LIMP and galactose, and catalytic amounts of ATP and glucose 1-phosphate, all inexpensive starting materials. After continuous circulation of the reaction mixture through the super-bead column for 48 h, 50% of the UMP was converted into UDP-galactose. The results show that de novo production of UDP-galactose on the super-bead column is more efficient than in solution because of the stability of the immobilized enzymes.

L12 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:843831 CAPLUS
DOCUMENT NUMBER: 136:4799
TITLE: Production of fucosylated carbohydrates by enzymatic fucosylation **synthesis** of **sugar nucleotides**; and in situ regeneration of GDP-fucose
INVENTOR(S): Wong, Chi-huey; Ichikawa, Yoshitaka; Shen, Gwo-jenn; Liu, Kun-chin
PATENT ASSIGNEE(S): Scripps Research Insitute, USA
SOURCE: U.S., 45 pp., Cont.-in-part of U.S. Ser. No. 910,612, abandoned. CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English

FAMILY ACC. NUM. COUNT: 7
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6319695	B1	20011120	US 1992-961076	19921014
WO 9308205	A1	19930429	WO 1992-US8789	19921015
W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
AU 9227854	A1	19930521	AU 1992-27854	19921015
AU 675209	B2	19970130		
JP 07500248	T2	19950112	JP 1992-507791	19921015
EP 642526	A1	19950315	EP 1992-921939	19921015
EP 642526	B1	19981223		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, SE				
HU 69791	A2	19950928	HU 1994-1072	19921015
AT 174925	E	19990115	AT 1992-921939	19921015
ES 2129458	T3	19990616	ES 1992-921939	19921015
FI 9401732	A	19940614	FI 1994-1732	19940414
NO 9401346	A	19940614	NO 1994-1346	19940414

PRIORITY APPLN. INFO.:
US 1991-777662 B2 19911015
US 1992-901260 B2 19920619
US 1992-910612 B2 19920708
US 1992-961076 A 19921014
WO 1992-US8789 A 19921015

OTHER SOURCE(S): CASREACT 136:4799

AB This invention contemplates improved methods of enzymic prodn. of carbohydrates esp. fucosylated carbohydrates. Improved **syntheses** of glycosyl 1- or 2-phosphates using both chem. and enzymic means are also contemplated. The phosphorylated glycosides are then used to produce **sugar nucleotides** that are in turn used as donor sugars for glycosylation of acceptor carbohydrates. Esp. preferred herein is the use of a disclosed method for fucosylation.

REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:634533 CAPLUS
DOCUMENT NUMBER: 136:242629
TITLE: The complete sequence of the 1,683-Kb pSymB megaplasmid from the N2-fixing endosymbiont Sinorhizobium meliloti
AUTHOR(S): Finan, Turlough M.; Weidner, Stefan; Wong, Kim; Buhrmester, Jens; Chain, Patrick; Vorholter, Frank J.; Hernandez-Lucas, Ismael; Becker, Anke; Cowie, Alison; Gouzy, Jerome; Golding, Brian; Puhler, Alfred
CORPORATE SOURCE: Department of Biology, McMaster University, Hamilton, ON, L8S 4K1, Can.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2001), 98(17), 9889-9894
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Anal. of the 1683,333-nt sequence of the pSymB megaplasmid from the symbiotic N2-fixing bacterium Sinorhizobium meliloti revealed that the replicon has a high gene d. with a total of 1570 protein-coding regions, with few insertion elements and regions duplicated elsewhere in the

genome. The only copies of an essential arg-tRNA gene and the minCDE genes are located on pSymB. Almost 20% of the pSymB sequence carries genes encoding solute uptake systems, most of which were of the ATP-binding cassette family. Many previously unsuspected genes involved in polysaccharide **biosynthesis** were identified and these, together with the two known distinct exopolysaccharide **synthesis** gene clusters, show that 14% of the pSymB sequence is dedicated to polysaccharide **synthesis**. Other recognizable gene clusters include many involved in catabolic activities such as protocatechuate utilization and phosphonate degrdn. The functions of these genes are consistent with the notion that pSymB plays a major role in the saprophytic competence of the bacteria in the soil environment.
REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS
RECORD. ALL CITATIONS AVAILABLE IN THE RE
FORMAT

L12 ANSWER 4 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2001:226644 SCISEARCH
THE GENUINE ARTICLE: 407CE
TITLE: **Sugar nucleotide** regeneration beads (superbeads): A versatile tool for the practical **synthesis** of oligosaccharides
AUTHOR: Chen X; Fang J W; Zhang J B; Liu Z Y; Shao J; Kowal P; Andreana P; Wang P G (Reprint)
CORPORATE SOURCE: Wayne State Univ, Dept Chem, Detroit, MI 48202 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, (7 MAR 2001) Vol. 123, No. 9, pp. 2081-2082.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.
ISSN: 0002-7863.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 35

L12 ANSWER 5 OF 14 MEDLINE
ACCESSION NUMBER: 2001214484 MEDLINE
DOCUMENT NUMBER: 21117088 PubMed ID: 11172001
TITLE: Physical and functional association of glycolipid N-acetyl-galactosaminyl and galactosyl transferases in the Golgi apparatus.
COMMENT: Comment in: Proc Natl Acad Sci U S A. 2001 Feb 13;98(4):1321-3
AUTHOR: Giraudo C G; Daniotti J L; Maccioni H J
CORPORATE SOURCE: Centro de Investigaciones en Quimica Biologica de Cordoba, Departamento de Quimica Biologica, Facultad de Ciencias Quimicas, Universidad Nacional de Cordoba, Ciudad Universitaria, 5000 Cordoba, Argentina.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Feb 13) 98 (4) 1625-30.
Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010425
Last Updated on STN: 20010425
Entered Medline: 20010419
AB Glycolipid glycosyltransferases catalyze the stepwise transfer of monosaccharides from **sugar nucleotides** to proper glycolipid acceptors. They are Golgi resident proteins that colocalize functionally in the organelle, but their intimate relationships are not known. Here, we show that the sequentially acting UDP-

GalNAc:lactosylceramide/GM3/GD3 beta-1,4-N-acetyl-galactosaminyltransferase and the UDP-Gal:GA2/GM2/[redacted] beta-1,3-galactosyltransferase associate physically in the cistal Golgi. Immunoprecipitation of the respective epitope-tagged versions expressed in transfected CHO-K1 cells resulted in their mutual coimmunoprecipitation. The immunocomplexes efficiently catalyze the two transfer steps leading to the **synthesis** of GM1 from exogenous GM3 in the presence of UDP-GalNAc and UDP-Gal. The N-terminal domains (cytosolic tail, transmembrane domain, and few amino acids of the stem region) of both enzymes are involved in the interaction because (i) they reproduce the coimmunoprecipitation behavior of the full-length enzymes, (ii) they compete with the full-length counterpart in both coimmunoprecipitation and GM1 **synthesis** experiments, and (iii) fused to the cyan and yellow fluorescent proteins, they localize these proteins to the Golgi membranes in an association close enough as to allow fluorescence resonance energy transfer between them. We suggest that these associations may improve the efficiency of glycolipid **synthesis** by channeling the intermediates from the position of product to the position of acceptor along the transfer steps.

L12 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:183001 CAPLUS
TITLE: Large-scale **synthesis** of carbohydrates for pharmaceutical development
AUTHOR(S): Zhang, Jianbo; Wu, Bingyuan; Liu, Ziye; Kowal, Premzek; Chen, Xi; Shao, Jun; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA
SOURCE: Current Organic Chemistry (2001), 5(12), 1169-1176
CODEN: CORCFE; ISSN: 1385-2728
PUBLISHER: Bentham Science Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The field of glycobiol. has recently enjoyed an enormous expansion boosted by new discoveries of the crit. functions that carbohydrates play in nature. Further research in this area and the entry of carbohydrates into the medical and pharmaceutical fields will undoubtedly require easy access to these mols. Recombinant glycosidases and glycosyltransferases, as well as their mutants and **fusion** proteins have already been applied in gram or even larger scale carbohydrate **synthesis**. Most efficient **synthetic** systems require expensive **sugar nucleotides** to be regenerated in situ. Solid support-immobilized **biosynthetic** enzymes and genetically engineered microorganisms have been demonstrated as viable and highly effective avenues to make carbohydrates. The efficiency of such systems makes them ideal for industry and should, at long last, make prodn. of complex carbohydrates economically feasible.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:351686 CAPLUS
DOCUMENT NUMBER: 133:3768
TITLE: Low cost enzymatic **biosynthesis** of oligosaccharides

INVENTOR(S): Defrees, Shawn; Johnson, Karl
PATENT ASSIGNEE(S): Neose Technologies, Inc., USA
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2

DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000029603	A2	20000525	WO 1999-US27599	19991118
WO 2000029603	A3	20001116		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000018261	A5	20000605	AU 2000-18261	19991118
EP 1131415	A2	20010912	EP 1999-961744	19991118
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 2002001831	A1	20020103	US 2001-757289	20010108

PRIORITY APPLN. INFO.:			
US 1998-109031P	P	19981118	
US 1998-109096P	P	19981119	
US 1999-442111	A1	19991117	
WO 1999-US27599	W	19991118	

AB This invention provides recombinant cells, reaction mixts., and methods for the enzymic **synthesis** of saccharides. The recombinant cells contain a heterologous gene that encodes a glycosyltransferase which catalyzes at least one step of the enzymic **synthesis**, as well a system for generating a nucleotide sugar that can serve as a substrate for the glycosyltransferase. The nucleotide sugar may be supplied or **synthesized** by an enzymic pathway comprising a **sugar nucleotide** regeneration cycle. The reaction mixt. may contain a second cell type producing a nucleotide as a substrate for the **sugar nucleotide** regeneration cycle, preferably by a nucleotide synthase gene. Use of **fusion** proteins of glycosyltransferase and nucleotide sugar synthase combined with the use of an enzyme for substrate sugar **synthesis** is described. Chem. or enzymic sulfation may be used for the **synthesis** of sulfated sugars. The recombinant cells, reaction mixts., and methods are useful for efficiently **synthesizing** a large variety of saccharides, including polysaccharides, oligosaccharides, glycoproteins and glycolipids, using relatively low-cost starting materials. **Synthesis** of 3'-sialyllactose using E. coli expressing a CMP-sialic acid **synthetase**/.alpha.2,3-sialyltransferase **fusion** protein is described. Optional use of bakers yeast to produce CTP used in the sialic acid cycle and substrate for CMP-sialic acid synthase is also described. **Synthesis** of 3'-sialyllactose using E. coli expressing a CMP-sialic acid **synthetase** /.alpha.2,3-sialyltransferase **fusion** protein, GlcNAc 2'-epimerase, and sialic acid aldolase to **synthesize** CMP-sialic acid from GlcNAc is also described. Variations of the method using Corynebacterium expressing a CMP-sialic acid **synthetase** /.alpha.2,3-sialyltransferase **fusion** protein and CTP-**synthetase** to produce the nucleotide, nucleotide sugar, and catalyzing sugar transfer to the acceptor saccharide is described. Finally, **synthesis** of trisaccharide Gal.alpha.1,3Gal.beta.1,4GlcNAc using Corynebacterium expressing

UDP-glucose pyrophosphorylase, UDP-glucose-4'-epimerase, .beta.1,4-galactosyltransferase, and .alpha.1,3-galactosyltransferase is described.

L12 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2
ACCESSION NUMBER: 2000:752980 CAPLUS
DOCUMENT NUMBER: 134:68004
TITLE: Changing the donor cofactor of bovine .alpha.1,3-galactosyltransferase by **fusion** with UDP-galactose 4-epimerase. More efficient biocatalysis for **synthesis** of .alpha.-Gal epitopes
AUTHOR(S): Chen, Xi; Liu, Ziye; Wang, Jianqiang; Fang, Jianwen; Fan, Hongni; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA
SOURCE: Journal of Biological Chemistry (2000), 275(41), 31594-31600
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Two **fusion** enzymes consisting of uridine diphosphogalactose 4-epimerase (UDP-galactose 4-epimerase, E.C. 5.1.3.2) and .alpha.1,3-galactosyltransferase (E.C. 2.4.1.151) with an N-terminal His6 tag and an intervening three-glycine linker were constructed by in-frame **fusion** of the Escherichia coli galE gene either to the 3' terminus (f1) or to the 5' terminus (f2) of a truncated bovine .alpha.1,3-galactosyltransferase gene, resp. Both **fusion** proteins were expressed in cell lysate as active, sol. forms as well as in inclusion bodies as improperly folded proteins. Both f1 and f2 were detd. to be homodimers, based on a single band obsd. at about 67 kDa in SDS-PAGE and on a single peak with a mol. mass around 140 kDa detd. by gel filtration chromatog. for each of the enzymes. Without altering the acceptor specificity of the transferase, the **fusion** with the epimerase changed the donor requirement of .alpha.1,3-galactosyltransferase from UDP-galactose to UDP-glucose and decreased the cost for the **synthesis** of biomedically important Gal.alpha.1,3Gal-terminated oligosaccharides by more than 40-fold. For enzymic **synthesis** of Gal.alpha.1,3Gal.beta.1,4Glc from UDP-glucose and lactose, the genetically fused enzymes f1 and f2 exhibited kinetic advantages with overall reaction rates that were 300 and 50%, resp., higher than that of the system contg. equal amts. of epimerase and galactosyltransferase. These results indicated that the active sites of the epimerase and the transferase in **fusion** enzymes were in proximity. The kinetic parameters suggested a random mechanism for the substrate binding of the .alpha.1,3-galactosyltransferase. This work demonstrated a general approach that **fusion** of a glycosyltransferase with an epimerase can change the required but expensive **sugar nucleotide** to a less expensive one.
REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:405071 CAPLUS
DOCUMENT NUMBER: 131:41527
TITLE: **Fusion** proteins for use in enzymatic **synthesis** of oligosaccharides
INVENTOR(S): Gilbert, Michel; Young, N. Martin; Wakarchuk, Warren W.
PATENT ASSIGNEE(S): National Research Council of Canada, Can.

SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9931224	A2	19990624	WO 1998-CA1180	19981215
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002034805	A1	20020321	US 1998-211691	19981214
CA 2315010	AA	19990624	CA 1998-2315010	19981215
AU 9917457	A1	19990705	AU 1999-17457	19981215
EP 1040186	A2	20001004	EP 1998-962154	19981215
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.: US 1997-69443P P 19971215
US 1998-211691 A 19981214
WO 1998-CA1180 W 19981215

AB This invention provides **fusion** polypeptides that include a glycosyltransferase catalytic domain and a catalytic domain from an accessory enzyme that is involved in making a substrate for a glycosyltransferase reaction. Nucleic acids that encode the **fusion** polypeptides are also provided, as are host cells for expressing the **fusion** polypeptides of the invention. Thus, using genes cloned from Neisseria meningitidis, a **fusion** protein which had both CMP-Neu5Ac **synthetase** and .alpha.-2,3-sialyltransferase activities was prepd. This **chimeric** enzyme was produced in high yields in Escherichia coli and functionally pure enzyme was obtained using a simple protocol. In small-scale enzymic **syntheses**, the **fusion** enzyme sialylated various oligosaccharide acceptors (branched and linear) with Neu5Ac as well as N-glycolyl- and N-propionyl-neuraminic acid in high yield. The **chimeric** enzyme was also used to produce .alpha.-2,3-sialyllactose at the 100 g scale using a **sugar nucleotide** cycle reaction, starting from lactose, sialic acid, PEP and catalytic amts. of ATP and CMP.

L12 ANSWER 10 OF 14 MEDLINE
ACCESSION NUMBER: 1999136149 MEDLINE
DOCUMENT NUMBER: 99136149 PubMed ID: 9949190
TITLE: Incorporation of 15N from ammonium into the N-linked oligosaccharides of an immunoadhesin glycoprotein expressed in Chinese hamster ovary cells.
AUTHOR: Gawlitzek M; Papac D I; Sliwkowski M B; Ryll T
CORPORATE SOURCE: Process Sciences, Genentech, Inc., 1 DNA Way, South San Francisco, CA, 94080-4990, USA.
SOURCE: GLYCOBIOLOGY, (1999 Feb) 9 (2) 125-31.
Journal code: BEL; 9104124. ISSN: 0959-6658.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990326
Last Updated on STN: 19990326
Entered Medline: 19990312

AB Elevated ammonium concentrations in the medium of cultivated cells have been shown to increase the intracellular levels of uridine-5'-diphospho-N-acetylglucosamine (UDP-GlcNAc) and uridine-5'-diphospho-N-acetylgalactosamine (UDP-GalNAc; Ryll et al., 1994). These **sugar nucleotides** are substrates for glycosyltransferases in the glycosylation pathway. In our experiments, recombinant Chinese hamster ovary cells producing an immunoadhesin glycoprotein (GP1-IgG) have been cultivated under controlled cell culture conditions in the presence of different ammonium concentrations.15N-Labeled ammonium chloride (15NH4Cl) was added exogenously to the cell culture media to determine if ammonium was incorporated into UDP-GlcNAc and cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-NANA) pools, and subsequently incorporated into GP1-IgG as N-linked glycans. The intracellular pools of UDP-activated hexosamines (UDP-GNAc) were followed during the time course of the experiment. To assess the extent of15NH4+incorporation into the glycans of GP1-IgG, the glycoprotein was first purified to homogeneity by protein A chromatography. Enzymatically released N-glycans were then analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. N-Glycans **synthesized** in the presence of15NH4Cl revealed an N-glycan-dependent increase in mass-to-charge of 2.5-4.8 Da. These results indicate that 60-70% of the total nitrogen containing monosaccharides had incorporated15N. Presumably,15NH4+was incorporated into GlcNAc and N-acetylneuraminic acid as proposed earlier (Ryll et al., 1994). This might be a universal and previously not described reaction in mammalian cells when exposed to nonphysiological but in cell culture commonly found concentrations of ammonium. The data presented here are of significance for glycoprotein production in mammalian cell culture, since it has been shown previously that elevated levels of UDP-activated hexosamines affect N-glycan characteristics such as branching and degree of amino sugar incorporation. In addition, our results demonstrate that isotope labeling in combination with MALDI-TOF-MS can be used as an alternate tool to radioactive labeling of sugar substrates in metabolic studies.

L12 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3

ACCESSION NUMBER: 1998:510061 CAPLUS
DOCUMENT NUMBER: 129:255694
TITLE: The **synthesis** of sialylated oligosaccharides using a CMP-Neu5Ac **synthetase** /sialyltransferase **fusion**
AUTHOR(S): Gilbert, Michel; Bayer, Robert; Cunningham, Anna-Marie; DeFrees, Shawn; Gao, Yinghong; Watson, David C.; Young, N. Martin; Wakarchuk, Warren W.
CORPORATE SOURCE: Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, K1A 0R6, Can.
SOURCE: Nat. Biotechnol. (1998), 16(8), 769-772
CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER: Nature America
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Large-scale enzymic **synthesis** of oligosaccharides, which contain terminal N-acetyl-neuraminic acid residues requires large amts. of the sialyltransferase and the corresponding **sugar-nucleotide synthetase**, which is required for the **synthesis** of the **sugar-nucleotide** donor, CMP-Neu5Ac. Using genes cloned from Neisseria meningitidis, we constructed a **fusion** protein that has both CMP-Neu5Ac **synthetase** and .alpha.-2,3-sialyltransferase activities. The **fusion** protein was produced in high yields (over 1200 U/L, measured using an .alpha.-2,3-sialyltransferase assay) in Escherichia coli and functionally pure enzyme could be obtained using a simple protocol. In small-scale enzymic **syntheses**, the **fusion** protein could sialylate various oligosaccharide acceptors (branched and linear) with N-acetyl-neuraminic

acid as well as N-glycolyl- and N-propionyl-neuraminic acid in high conversion yield. The **fusion** protein was also used to produce .alpha.-2,3-sialyllactose at the 100 g scale using a **sugar nucleotide** cycle reaction, starting from lactose, sialic acid, phosphoenolpyruvate, and catalytic amts. of ATP and CMP.

L12 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:473637 CAPLUS
DOCUMENT NUMBER: 127:80242
TITLE: **Synthesis** of hybrid molecules of two segments containing peptide and nonpeptide portions
INVENTOR(S): De Crecy Lagard, Valerie; Marliere, Philippe; Saurin, William
PATENT ASSIGNEE(S): Institut Pasteur, Fr.
SOURCE: Fr. Demande, 25 pp.
CODEN: FRXXBL
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2739628	A1	19970411	FR 1995-11730	19951005
FR 2739628	B1	19971226		

AB The **synthesis** of hybrid mols. consisting of the condensation of .gtoreq.2 sequences of 2 different chem. types is disclosed. The types may be amino acids or their derivs., peptides, prosthetic groups, **sugars**, **nucleotides**, fatty acids, natural polymers, or their fragments. These hybrid mols. may be utilized in the therapeutic, food, agronomy, or plastics industries.

INDEX 'ADISALERTS, ADISINSIGHT, ADISNEWS, AGRICOLA, ANABSTR, AQUASCI, BIOBUSINESS, BIOCOMMERCE, BIOSIS, BIOTECHABS, BIOTECHDS, BIOTECHNO, CABA, CANCERLIT, CAPLUS, CEABA-VTB, CEN, CIN, CONFSCI, CROPB, CROPU, DDFB, DDFU, DGENE, DRUGB, DRUGLAUNCH, DRUGMONOG2, ...' ENTERED AT 14:29:40 ON 07 MAY 2002

SEA	ENZYME
8844	FILE ADISALERTS
3380	FILE ADISINSIGHT
1771	FILE ADISNEWS
82992	FILE AGRICOLA
12494	FILE ANABSTR
19547	FILE AQUASCI
53313	FILE BIOBUSINESS
12742	FILE BIOCOMMERCE
642440	FILE BIOSIS
92228	FILE BIOTECHABS
92228	FILE BIOTECHDS
300806	FILE BIOTECHNO
169044	FILE CABA
99358	FILE CANCERLIT
798553	FILE CAPLUS
27932	FILE CEABA-VTB
1524	FILE CEN
6548	FILE CIN
10028	FILE CONFSCI
5084	FILE CROPB
5013	FILE CROPU
29199	FILE DDFB
38582	FILE DDFU
148103	FILE DGENE
29199	FILE DRUGB
371	FILE DRUGLAUNCH
72	FILE DRUGMONOG2
663	FILE DRUGNL
55034	FILE DRUGU
715	FILE DRUGUPDATES
3256	FILE EMBAL
627925	FILE EMBASE
163249	FILE ESBIOBASE
166	FILE FOMAD
1043	FILE FOREGE
31935	FILE FROSTI
45036	FILE FSTA
72053	FILE GENBANK
1860	FILE HEALSAFE
24725	FILE IFIPAT
187536	FILE JICST-EPLUS
1105	FILE KOSMET
171354	FILE LIFESCI
338	FILE MEDICONF
596249	FILE MEDLINE
16437	FILE NIOSHTIC
11537	FILE NTIS
5790	FILE OCEAN
422732	FILE PASCAL
7244	FILE PHAR
28	FILE PHIC

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            5705   FILE PHIN
           30501   FILE PROMT
          367891   FILE SCISEARCH
              78   FILE SYNTHLINE
          286015   FILE TOXCENTER
          106258   FILE USPATFULL
             279   FILE USPAT2
           58240   FILE WPIDS
           58240   FILE WPINDEX
L1              QUE ENZYME
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     FILE 'CAPLUS, BIOSIS, EMBASE, MEDLINE, SCISEARCH, BIOTECHNO' ENTERED AT
     14:32:36 ON 07 MAY 2002
L2         57169 S L1 AND (FUSION) OR (DUAL(W)FUNCTION)
L3           275 S L2 AND GLYCOSYLTRANSFERASE
L4            10 S L3 AND NUCLEOTIDE(W)SUGAR
L5             5 DUP REM L4 (5 DUPLICATES REMOVED)
L6            35 S L3 AND CHIMER?
L7            27 DUP REM L6 (8 DUPLICATES REMOVED)

=> s (sugar(w)nucleotide) or oligosaccahride

L8         2441 (SUGAR(W) NUCLEOTIDE) OR OLIGOSACCAHRIDE

=> s l8 and (synthe?) or (biosynthe?)

L9       895222 L8 AND (SYNTHE?) OR (BIOSYNTHE?)

=> s l8 and (synthe? or biosynthe?)

L10        1459 L8 AND (SYNTHE? OR BIOSYNTHE?)

=> s l10 and (hybrid(w)enzyme OR chimer? or fusion)

L11          27 L10 AND (HYBRID(W) ENZYME OR CHIMER? OR FUSION)

=> dup rem l11

PROCESSING COMPLETED FOR L11
L12           14 DUP REM L11 (13 DUPLICATES REMOVED)

=> d l12 ibib ab 1-12


L12  ANSWER 1 OF 14  SCISEARCH  COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER:    2002:308856  SCISEARCH
THE GENUINE ARTICLE: 538TL
TITLE:               Combined biosynthetic pathway for de novo
                     production of UDP-galactose: Catalysis with multiple
                     enzymes immobilized on agarose beads
AUTHOR:              Liu Z Y; Zhang J B; Chen X; Wang P G (Reprint)
CORPORATE SOURCE:    Wayne State Univ, Dept Chem, Detroit, MI 48202 USA
                     (Reprint); Neose Technol Ltd, Hursham, PA 19044 USA
COUNTRY OF AUTHOR:   USA
SOURCE:              CHEMBIOCHEM, (2 APR 2002) Vol. 3, No. 4, pp. 348-355.
                     Publisher: WILEY-V C H VERLAG GMBH, PO BOX 10 11 61,
                     D-69451 WEINHEIM, GERMANY.
                     ISSN: 1439-4227.
DOCUMENT TYPE:       Article; Journal
LANGUAGE:            English
REFERENCE COUNT:     46
                    *ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS*
AB       Regeneration of sugar nucleotides is a critical
     step in the biosynthetic pathway for the formation of
     oligosaccharides. To alleviate the difficulties in the production of
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sugar nucleotides, we have developed a method to produce uridine diphospha[redacted] galactose (UDP-galactose). The[redacted]mbined **biosynthetic** pathway, which involves seven enzymes, is composed of three parts: i) the main pathway to form UDP-galoctose from galactose, with the enzymes galactokinase, galactose-1-phosphate uridyltransferase, UDP-glucose pyrophosphorylase, and inorganic pyrophosphotase, 11) the uridine triphosphate supply pathway catalyzed by uridine monophosphate (UMP) kinase and nucleotide diphosphate kinase, and iii) the adenosine triphosphate (ATP) regeneration pathway catalyzed by polyphosphate kinase with polyphosphate added as an energy resource. All of the enzymes were expressed individually and immobilized through their hexahistidine tags onto nickel agarose beads ("super beads"). The reaction requires a stoichlometric amount of LIMP and galactose, and catalytic amounts of ATP and glucose 1-phosphate, all inexpensive starting materials. After continuous circulation of the reaction mixture through the super-bead column for 48 h, 50% of the UMP was converted into UDP-galactose. The results show that de novo production of UDP-galactose on the super-bead column is more efficient than in solution because of the stability of the immobilized enzymes.

L12 ANSWER 2 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:843831 CAPLUS
DOCUMENT NUMBER: 136:4799
TITLE: Production of fucosylated carbohydrates by enzymatic fucosylation **synthesis** of **sugar nucleotides**; and in situ regeneration of GDP-fucose
INVENTOR(S): Wong, Chi-huey; Ichikawa, Yoshitaka; Shen, Gwo-jenn; Liu, Kun-chin
PATENT ASSIGNEE(S): Scripps Research Insitute, USA
SOURCE: U.S., 45 pp., Cont.-in-part of U.S. Ser. No. 910,612, abandoned.
CODEN: USXXAM
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 7
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 6319695	B1	20011120	US 1992-961076	19921014
WO 9308205	A1	19930429	WO 1992-US8789	19921015
W: AU, BB, BG, BR, CA, CS, FI, HU, JP, KP, KR, LK, MG, MN, MW, NO, PL, RO, RU, SD				
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, SN, TD, TG				
AU 9227854	A1	19930521	AU 1992-27854	19921015
AU 675209	B2	19970130		
JP 07500248	T2	19950112	JP 1992-507791	19921015
EP 642526	A1	19950315	EP 1992-921939	19921015
EP 642526	B1	19981223		
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LI, LU, MC, NL, SE				
HU 69791	A2	19950928	HU 1994-1072	19921015
AT 174925	E	19990115	AT 1992-921939	19921015
ES 2129458	T3	19990616	ES 1992-921939	19921015
FI 9401732	A	19940614	FI 1994-1732	19940414
NO 9401346	A	19940614	NO 1994-1346	19940414

PRIORITY APPLN. INFO.:
US 1991-777662 B2 19911015
US 1992-901260 B2 19920619
US 1992-910612 B2 19920708
US 1992-961076 A 19921014
WO 1992-US8789 A 19921015

OTHER SOURCE(S): CASREACT 136:4799
AB This invention contemplates improved methods of enzymic prodn. of carbohydrates esp. fucosylated carbohydrates. Improved **syntheses** of glycosyl 1- or 2-phosphates using both chem. and enzymic means are also

contemplated. The phosphorylated glycosides are then used to produce **sugar nucleotides** that are in turn used as donor sugars for glycosylation of acceptor carbohydrates. Esp. preferred herein is the use of a disclosed method for fucosylation.
REFERENCE COUNT: 37 THERE ARE 37 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 3 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2001:634533 CAPLUS
DOCUMENT NUMBER: 136:242629
TITLE: The complete sequence of the 1,683-Kb pSymB megaplasmid from the N2-fixing endosymbiont Sinorhizobium meliloti
AUTHOR(S): Finan, Turlough M.; Weidner, Stefan; Wong, Kim; Buhrmester, Jens; Chain, Patrick; Vorholter, Frank J.; Hernandez-Lucas, Ismael; Becker, Anke; Cowie, Alison; Gouzy, Jerome; Golding, Brian; Puhler, Alfred
CORPORATE SOURCE: Department of Biology, McMaster University, Hamilton, ON, L8S 4K1, Can.
SOURCE: Proceedings of the National Academy of Sciences of the United States of America (2001), 98(17), 9889-9894
CODEN: PNASA6; ISSN: 0027-8424
PUBLISHER: National Academy of Sciences
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Anal. of the 1683,333-nt sequence of the pSymB megaplasmid from the symbiotic N2-fixing bacterium Sinorhizobium meliloti revealed that the replicon has a high gene d. with a total of 1570 protein-coding regions, with few insertion elements and regions duplicated elsewhere in the genome. The only copies of an essential arg-tRNA gene and the minCDE genes are located on pSymB. Almost 20% of the pSymB sequence carries genes encoding solute uptake systems, most of which were of the ATP-binding cassette family. Many previously unsuspected genes involved in polysaccharide **biosynthesis** were identified and these, together with the two known distinct exopolysaccharide **synthesis** gene clusters, show that 14% of the pSymB sequence is dedicated to polysaccharide **synthesis**. Other recognizable gene clusters include many involved in catabolic activities such as protocatechuate utilization and phosphonate degrdn. The functions of these genes are consistent with the notion that pSymB plays a major role in the saprophytic competence of the bacteria in the soil environment.
REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 4 OF 14 SCISEARCH COPYRIGHT 2002 ISI (R)
ACCESSION NUMBER: 2001:226644 SCISEARCH
THE GENUINE ARTICLE: 407CE
TITLE: **Sugar nucleotide** regeneration beads (superbeads): A versatile tool for the practical **synthesis** of oligosaccharides
AUTHOR: Chen X; Fang J W; Zhang J B; Liu Z Y; Shao J; Kowal P; Andreana P; Wang P G (Reprint)
CORPORATE SOURCE: Wayne State Univ, Dept Chem, Detroit, MI 48202 USA (Reprint)
COUNTRY OF AUTHOR: USA
SOURCE: JOURNAL OF THE AMERICAN CHEMICAL SOCIETY, (7 MAR 2001) Vol. 123, No. 9, pp. 2081-2082.
Publisher: AMER CHEMICAL SOC, 1155 16TH ST, NW, WASHINGTON, DC 20036 USA.

ISSN: 0002-7863.
DOCUMENT TYPE: Article; Journal
LANGUAGE: English
REFERENCE COUNT: 35

L12 ANSWER 5 OF 14 MEDLINE
ACCESSION NUMBER: 2001214484 MEDLINE
DOCUMENT NUMBER: 21117088 PubMed ID: 11172001
TITLE: Physical and functional association of glycolipid N-acetyl-galactosaminyl and galactosyl transferases in the Golgi apparatus.
COMMENT: Comment in: Proc Natl Acad Sci U S A. 2001 Feb 13;98(4):1321-3
AUTHOR: Giraudo C G; Daniotti J L; Maccioni H J
CORPORATE SOURCE: Centro de Investigaciones en Quimica Biologica de Cordoba, Departamento de Quimica Biologica, Facultad de Ciencias Quimicas, Universidad Nacional de Cordoba, Ciudad Universitaria, 5000 Cordoba, Argentina.
SOURCE: PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (2001 Feb 13) 98 (4) 1625-30. Journal code: PV3; 7505876. ISSN: 0027-8424.
PUB. COUNTRY: United States
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 200104
ENTRY DATE: Entered STN: 20010425
Last Updated on STN: 20010425
Entered Medline: 20010419

AB Glycolipid glycosyltransferases catalyze the stepwise transfer of monosaccharides from **sugar nucleotides** to proper glycolipid acceptors. They are Golgi resident proteins that colocalize functionally in the organelle, but their intimate relationships are not known. Here, we show that the sequentially acting UDP-GalNAc:lactosylceramide/GM3/GD3 beta-1,4-N-acetyl-galactosaminyltransferase and the UDP-Gal:GA2/GM2/GD2 beta-1,3-galactosyltransferase associate physically in the distal Golgi. Immunoprecipitation of the respective epitope-tagged versions expressed in transfected CHO-K1 cells resulted in their mutual coimmunoprecipitation. The immunocomplexes efficiently catalyze the two transfer steps leading to the **synthesis** of GM1 from exogenous GM3 in the presence of UDP-GalNAc and UDP-Gal. The N-terminal domains (cytosolic tail, transmembrane domain, and few amino acids of the stem region) of both enzymes are involved in the interaction because (i) they reproduce the coimmunoprecipitation behavior of the full-length enzymes, (ii) they compete with the full-length counterpart in both coimmunoprecipitation and GM1 **synthesis** experiments, and (iii) fused to the cyan and yellow fluorescent proteins, they localize these proteins to the Golgi membranes in an association close enough as to allow fluorescence resonance energy transfer between them. We suggest that these associations may improve the efficiency of glycolipid **synthesis** by channeling the intermediates from the position of product to the position of acceptor along the transfer steps.

L12 ANSWER 6 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 1
ACCESSION NUMBER: 2002:183001 CAPLUS
TITLE: Large-scale **synthesis** of carbohydrates for pharmaceutical development
AUTHOR(S): Zhang, Jianbo; Wu, Bingyuan; Liu, Ziye; Kowal, Premzek; Chen, Xi; Shao, Jun; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University,

Detroit, MI, 48202, USA
SOURCE: Current Organic Chemistry (200[redacted] 5(12), 1169-1176
CODEN: CORCFE; ISSN: 1385-2728[redacted]
PUBLISHER: Bentham Science Publishers
DOCUMENT TYPE: Journal
LANGUAGE: English
AB The field of glycobiol. has recently enjoyed an enormous expansion boosted by new discoveries of the crit. functions that carbohydrates play in nature. Further research in this area and the entry of carbohydrates into the medical and pharmaceutical fields will undoubtedly require easy access to these mols. Recombinant glycosidases and glycosyltransferases, as well as their mutants and **fusion** proteins have already been applied in gram or even larger scale carbohydrate **synthesis**. Most efficient **synthetic** systems require expensive **sugar nucleotides** to be regenerated in situ. Solid support-immobilized **biosynthetic** enzymes and genetically engineered microorganisms have been demonstrated as viable and highly effective avenues to make carbohydrates. The efficiency of such systems makes them ideal for industry and should, at long last, make prodn. of complex carbohydrates economically feasible.
REFERENCE COUNT: 46 THERE ARE 46 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 7 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 2000:351686 CAPLUS
DOCUMENT NUMBER: 133:3768
TITLE: Low cost enzymatic **biosynthesis** of oligosaccharides
INVENTOR(S): Defrees, Shawn; Johnson, Karl
PATENT ASSIGNEE(S): Neose Technologies, Inc., USA
SOURCE: PCT Int. Appl., 103 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2000029603	A2	20000525	WO 1999-US27599	19991118
WO 2000029603	A3	20001116		
W: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
AU 2000018261	A5	20000605	AU 2000-18261	19991118
EP 1131415	A2	20010912	EP 1999-961744	19991118
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO				
US 2002001831	A1	20020103	US 2001-757289	20010108

PRIORITY APPLN. INFO.:
US 1998-109031P P 19981118
US 1998-109096P P 19981119
US 1999-442111 A1 19991117
WO 1999-US27599 W 19991118

AB This invention provides recombinant cells, reaction mixts., and methods

for the enzymic **synthesis** of saccharides. The recombinant cells contain a heterol[redacted]us gene that encodes a glycosy[redacted]ransferase which catalyzes at leas[redacted]one step of the enzymic **synthesis**, as well a system for generating a nucleotide sugar that can serve as a substrate for the glycosyltransferase. The nucleotide sugar may be supplied or **synthesized** by an enzymic pathway comprising a **sugar nucleotide** regeneration cycle. The reaction mixt. may contain a second cell type producing a nucleotide as a substrate for the **sugar nucleotide** regeneration cycle, preferably by a nucleotide synthase gene. Use of **fusion** proteins of glycosyltransferase and nucleotide sugar synthase combined with the use of an enzyme for substrate sugar **synthesis** is described. Chem. or enzymic sulfation may be used for the **synthesis** of sulfated sugars. The recombinant cells, reaction mixts., and methods are useful for efficiently **synthesizing** a large variety of saccharides, including polysaccharides, oligosaccharides, glycoproteins and glycolipids, using relatively low-cost starting materials. **Synthesis** of 3'-sialyllactose using E. coli expressing a CMP-sialic acid **synthetase**/.alpha.2,3-sialyltransferase **fusion** protein is described. Optional use of bakers yeast to produce CTP used in the sialic acid cycle and substrate for CMP-sialic acid synthase is also described. **Synthesis** of 3'-sialyllactose using E. coli expressing a CMP-sialic acid **synthetase** /.alpha.2,3-sialyltransferase **fusion** protein, GlcNAc 2'-epimerase, and sialic acid aldolase to **synthesize** CMP-sialic acid from GlcNAc is also described. Variations of the method using Corynebacterium expressing a CMP-sialic acid **synthetase** /.alpha.2,3-sialyltransferase **fusion** protein and CTP-**synthetase** to produce the nucleotide, nucleotide sugar, and catalyzing sugar transfer to the acceptor saccharide is described. Finally, **synthesis** of trisaccharide Gal.alpha.1,3Gal.beta.1,4GlcNAc using Corynebacterium expressing UDP-glucose pyrophosphorylase, UDP-glucose-4'-epimerase, .beta.1,4-galactosyltransferase, and .alpha.1,3-galactosyltransferase is described.

L12 ANSWER 8 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 2

ACCESSION NUMBER: 2000:752980 CAPLUS
DOCUMENT NUMBER: 134:68004
TITLE: Changing the donor cofactor of bovine .alpha.1,3-galactosyltransferase by **fusion** with UDP-galactose 4-epimerase. More efficient biocatalysis for **synthesis** of .alpha.-Gal epitopes
AUTHOR(S): Chen, Xi; Liu, Ziye; Wang, Jianqiang; Fang, Jianwen; Fan, Hongni; Wang, Peng George
CORPORATE SOURCE: Department of Chemistry, Wayne State University, Detroit, MI, 48202, USA
SOURCE: Journal of Biological Chemistry (2000), 275(41), 31594-31600
CODEN: JBCHA3; ISSN: 0021-9258
PUBLISHER: American Society for Biochemistry and Molecular Biology
DOCUMENT TYPE: Journal
LANGUAGE: English

AB Two **fusion** enzymes consisting of uridine diphosphogalactose 4-epimerase (UDP-galactose 4-epimerase, E.C. 5.1.3.2) and .alpha.1,3-galactosyltransferase (E.C. 2.4.1.151) with an N-terminal His6 tag and an intervening three-glycine linker were constructed by in-frame **fusion** of the Escherichia coli galE gene either to the 3' terminus (f1) or to the 5' terminus (f2) of a truncated bovine .alpha.1,3-galactosyltransferase gene, resp. Both **fusion** proteins were expressed in cell lysate as active, sol. forms as well as in inclusion bodies as improperly folded proteins. Both f1 and f2 were detd. to be

homodimers, based on a single band obsd. at about 67 kDa in SDS-PAGE and on a single peak with a mol. mass around 140 kDa d[illegible]. by gel filtration chromatog. for each of the enzymes. Without altering the acceptor specificity of the transferase, the **fusion** with the epimerase changed the donor requirement of .alpha.1,3-galactosyltransferase from UDP-galactose to UDP-glucose and decreased the cost for the **synthesis** of biomedically important Gal.alpha.1,3Gal-terminated oligosaccharides by more than 40-fold. For enzymic **synthesis** of Gal.alpha.1,3Gal.beta.1,4Glc from UDP-glucose and lactose, the genetically fused enzymes f1 and f2 exhibited kinetic advantages with overall reaction rates that were 300 and 50%, resp., higher than that of the system contg. equal amts. of epimerase and galactosyltransferase. These results indicated that the active sites of the epimerase and the transferase in **fusion** enzymes were in proximity. The kinetic parameters suggested a random mechanism for the substrate binding of the .alpha.1,3-galactosyltransferase. This work demonstrated a general approach that **fusion** of a glycosyltransferase with an epimerase can change the required but expensive **sugar nucleotide** to a less expensive one.

REFERENCE COUNT: 38 THERE ARE 38 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L12 ANSWER 9 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1999:405071 CAPLUS
DOCUMENT NUMBER: 131:41527
TITLE: **Fusion** proteins for use in enzymatic **synthesis** of oligosaccharides
INVENTOR(S): Gilbert, Michel; Young, N. Martin; Wakarchuk, Warren W.
PATENT ASSIGNEE(S): National Research Council of Canada, Can.
SOURCE: PCT Int. Appl., 63 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9931224	A2	19990624	WO 1998-CA1180	19981215
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
US 2002034805	A1	20020321	US 1998-211691	19981214
CA 2315010	AA	19990624	CA 1998-2315010	19981215
AU 9917457	A1	19990705	AU 1999-17457	19981215
EP 1040186	A2	20001004	EP 1998-962154	19981215
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRIORITY APPLN. INFO.:
US 1997-69443P P 19971215
US 1998-211691 A 19981214
WO 1998-CA1180 W 19981215

AB This invention provides **fusion** polypeptides that include a glycosyltransferase catalytic domain and a catalytic domain from an accessory enzyme that is involved in making a substrate for a glycosyltransferase reaction. Nucleic acids that encode the **fusion** polypeptides are also provided, as are host cells for

expressing the **fusion** polypeptides of the invention. Thus, using genes clone[redacted]from Neisseria meningitidis, a [redacted]**sion** protein which had both CMP-Neu5Ac **synthetase** and .alpha.-2,3-sialyltransferase activities was prepd. This **chimeric** enzyme was produced in high yields in Escherichia coli and functionally pure enzyme was obtained using a simple protocol. In small-scale enzymic **syntheses**, the **fusion** enzyme sialylated various oligosaccharide acceptors (branched and linear) with Neu5Ac as well as N-glycolyl- and N-propionyl-neuraminic acid in high yield. The **chimeric** enzyme was also used to produce .alpha.-2,3-sialyllactose at the 100 g scale using a **sugar nucleotide** cycle reaction, starting from lactose, sialic acid, PEP and catalytic amts. of ATP and CMP.

L12 ANSWER 10 OF 14 MEDLINE
ACCESSION NUMBER: 1999136149 MEDLINE
DOCUMENT NUMBER: 99136149 PubMed ID: 9949190
TITLE: Incorporation of 15N from ammonium into the N-linked oligosaccharides of an immunoadhesin glycoprotein expressed in Chinese hamster ovary cells.
AUTHOR: Gawlitzek M; Papac D I; Sliwkowski M B; Ryll T
CORPORATE SOURCE: Process Sciences, Genentech, Inc., 1 DNA Way, South San Francisco, CA, 94080-4990, USA.
SOURCE: GLYCOBIOLOGY, (1999 Feb) 9 (2) 125-31.
Journal code: BEL; 9104124. ISSN: 0959-6658.
PUB. COUNTRY: ENGLAND: United Kingdom
Journal; Article; (JOURNAL ARTICLE)
LANGUAGE: English
FILE SEGMENT: Priority Journals
ENTRY MONTH: 199903
ENTRY DATE: Entered STN: 19990326
Last Updated on STN: 19990326
Entered Medline: 19990312

AB Elevated ammonium concentrations in the medium of cultivated cells have been shown to increase the intracellular levels of uridine-5'-diphospho-N-acetylglucosamine (UDP-GlcNAc) and uridine-5'-diphospho-N-acetylgalactosamine (UDP-GalNAc; Ryll et al., 1994). These **sugar nucleotides** are substrates for glycosyltransferases in the glycosylation pathway. In our experiments, recombinant Chinese hamster ovary cells producing an immunoadhesin glycoprotein (GP1-IgG) have been cultivated under controlled cell culture conditions in the presence of different ammonium concentrations.15N-Labeled ammonium chloride (15NH4Cl) was added exogenously to the cell culture media to determine if ammonium was incorporated into UDP-GlcNAc and cytidine-5'-monophospho-N-acetylneuraminic acid (CMP-NANA) pools, and subsequently incorporated into GP1-IgG as N-linked glycans. The intracellular pools of UDP-activated hexosamines (UDP-GNAc) were followed during the time course of the experiment. To assess the extent of15NH4+incorporation into the glycans of GP1-IgG, the glycoprotein was first purified to homogeneity by protein A chromatography. Enzymatically released N-glycans were then analyzed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. N-Glycans **synthesized** in the presence of15NH4Cl revealed an N-glycan-dependent increase in mass-to-charge of 2.5-4.8 Da. These results indicate that 60-70% of the total nitrogen containing monosaccharides had incorporated15N. Presumably,15NH4+was incorporated into GlcNAc and N-acetylneuraminic acid as proposed earlier (Ryll et al., 1994). This might be a universal and previously not described reaction in mammalian cells when exposed to nonphysiological but in cell culture commonly found concentrations of ammonium. The data presented here are of significance for glycoprotein production in mammalian cell culture, since it has been shown previously that elevated levels of UDP-activated hexosamines affect N-glycan characteristics such as branching and degree

of amino sugar incorporation. In addition, our results demonstrate that isotope labeling [redacted] combination with MALDI-TOF-MS [redacted] be used as an alternate tool to radioactive labeling of sugar substrates in metabolic studies.

L12 ANSWER 11 OF 14 CAPLUS COPYRIGHT 2002 ACS DUPLICATE 3
ACCESSION NUMBER: 1998:510061 CAPLUS
DOCUMENT NUMBER: 129:255694
TITLE: The **synthesis** of sialylated oligosaccharides using a CMP-Neu5Ac **synthetase** /sialyltransferase **fusion**
AUTHOR(S): Gilbert, Michel; Bayer, Robert; Cunningham, Anna-Marie; DeFrees, Shawn; Gao, Yinghong; Watson, David C.; Young, N. Martin; Wakarchuk, Warren W.
CORPORATE SOURCE: Institute for Biological Sciences, National Research Council of Canada, Ottawa, ON, K1A 0R6, Can.
SOURCE: Nat. Biotechnol. (1998), 16(8), 769-772
CODEN: NABIF9; ISSN: 1087-0156
PUBLISHER: Nature America
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Large-scale enzymic **synthesis** of oligosaccharides, which contain terminal N-acetyl-neuraminic acid residues requires large amts. of the sialyltransferase and the corresponding **sugar-nucleotide synthetase**, which is required for the **synthesis** of the **sugar-nucleotide** donor, CMP-Neu5Ac. Using genes cloned from Neisseria meningitidis, we constructed a **fusion** protein that has both CMP-Neu5Ac **synthetase** and .alpha.-2,3-sialyltransferase activities. The **fusion** protein was produced in high yields (over 1200 U/L, measured using an .alpha.-2,3-sialyltransferase assay) in Escherichia coli and functionally pure enzyme could be obtained using a simple protocol. In small-scale enzymic **syntheses**, the **fusion** protein could sialylate various oligosaccharide acceptors (branched and linear) with N-acetyl-neuraminic acid as well as N-glycolyl- and N-propionyl-neuraminic acid in high conversion yield. The **fusion** protein was also used to produce .alpha.-2,3-sialyllactose at the 100 g scale using a **sugar nucleotide** cycle reaction, starting from lactose, sialic acid, phosphoenolpyruvate, and catalytic amts. of ATP and CMP.

L12 ANSWER 12 OF 14 CAPLUS COPYRIGHT 2002 ACS
ACCESSION NUMBER: 1997:473637 CAPLUS
DOCUMENT NUMBER: 127:80242
TITLE: **Synthesis** of hybrid molecules of two segments containing peptide and nonpeptide portions
INVENTOR(S): De Crecy Lagard, Valerie; Marliere, Philippe; Saurin, William
PATENT ASSIGNEE(S): Institut Pasteur, Fr.
SOURCE: Fr. Demande, 25 pp.
CODEN: FRXXBL
DOCUMENT TYPE: Patent
LANGUAGE: French
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
FR 2739628	A1	19970411	FR 1995-11730	19951005
FR 2739628	B1	19971226		

AB The **synthesis** of hybrid mols. consisting of the condensation of .gtoreq.2 sequences of 2 different chem. types is disclosed. The types may be amino acids or their derivs., peptides, prosthetic groups, **sugars**, **nucleotides**, fatty acids, natural polymers, or their fragments. These hybrid mols. may be utilized in the therapeutic, food, agronomy, or plastics industries.